

Figure 1

Shuffle Rubisco to improve its specificity Form I enzyme (*Cyanobacteria*)

Construct a transformation vector for *Synechocystis* sp. PCC 6803: (5' region-Kan^R-promoter-rbcL-rbcX-rbcS-Spec^R-3' region)

Clone and synthesize genes (*Synechocystis*,
Cynechoccocus, *Anabaena*)

Shuffle rbcL

Clone shuffled rbcL into transformation
vector (shuffled library)

Transform the library to *Synechocystis*

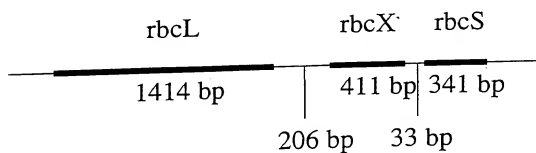
Select transformants by Kan^R/Spec^R and
photoautotrophic growth

Screen improved Rubisco by growing transformants
under elevated O₂ and/or high temperature (35-45 °C)

Enzyme purification and assay:
CO₂/O₂ specificity, specific activity

Figure 2

A *Synechocystis* Rubisco gene structure



B *Synechocystis* transformation - homologous replacement

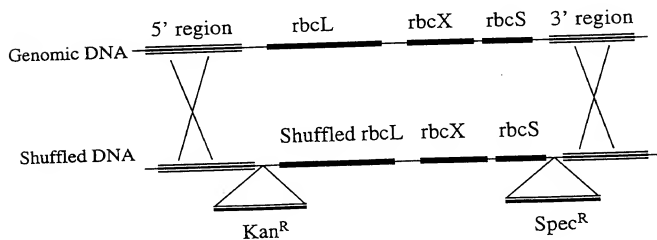


Figure 3

Shuffle Rubisco to improve its specificity Form II enzyme

Synthesize

Genes) (several form II Rubisco sequences are available:
Rhodospirillum rubrum, *Rhodobacter capsulatus*,
Thiobacillus denitrificans, *Riftia pachyptila endosymbiont*)

Shuffled gene

Clone into expression vector

Transform to a Rubisco deletion
strain of *R. rubrum*

Photoautotrophically screen improved Rubisco colonies
under aerobic condition with different O₂ concentrations

Enzyme purification and assay:
CO₂/O₂ specificity, specific activity

Figure 4
Shuffle Rubisco to improve its specificity
Form II enzyme
***E. coli* screening system (prk)**

Synthesize Genes (several form II Rubisco sequences are available: *Rhodospirillum rubrum*, *Rhodobacter capsulatus*, *Thiobacillus denitrificans*, *Riftia pachyptila endosymbiont*)

↓

Shuffled gene

↓

Clone into *E. coli* expression vector

↓

Transform to *E. coli* with prk

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Screen functional Rubisco colonies by $^{14}\text{CO}_2$ incorporation

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Enzyme purification and assay:
 CO_2/O_2 specificity, specific activity

Figure 5

Shuffle high specificity marine
Rubisco rbcL/S operon

